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Pages (Including Cover):	8	**Date:**	May 12, 2009
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From:	Homer W. Faucett, III		
Phone:	(317) 236-2120		
Subject:	Re:	Serial No.:	10/531,966
		Title:	AMPLICON MELTING ANALYSIS WITH SATURATION DYES
		Inventor:	WITTWER, Carl T., et al.
		Filed:	April 20, 2005
		Confirmation No.:	8958
		Our File No.:	P00950-US-01 (21932.0023)

Message:

Per our discussion, I am attaching a copy of our proposed claims for an Examiner's Amendment. Please call me at (317) 236-2120 to discuss.

Sincerely,

Homer W. Faucett, III

Client / Matter: 21932.0023 Job Code (Fax Center Use Only): 9801

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In the Claims.

Please amend the claims as follows:

1-19. (Cancelled)

20. (Previously Presented) The method of claim 24 wherein the target nucleic acid comprises a single nucleotide polymorphism, and the identifying step comprises identifying resultant heteroduplexes and homoduplexes.

21-22. (Cancelled)

23. (Previously Presented) The method of claim 24 wherein the method comprises mutation scanning, and the method further comprises repeating the amplifying and monitoring steps on second sample to obtain a second melting curve, and comparing the melting curves.

24. (Currently Amended) A method of PCR analysis comprising the steps of:

mixing a dsDNA binding dye having a percent saturation of at least 90% with a sample comprising a selected target nucleic acid and primers configured for amplifying the selected target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye, and

monitoring fluorescence of the dsDNA binding dye, wherein the monitoring step comprises

melting the amplified target nucleic acid to generate a melting curve, ~~and~~

~~identifying the genotype using a shape of the melting curve~~

repeating the mixing, amplifying, melting, and generating a melting curve steps with at least one additional target nucleic acid, and

comparing the melting curves.

wherein the melting curve for the selected target nucleic acid is selected as the standard and is plotted as standard across melting temperatures and the melting curve for each additional target nucleic acid is plotted as a difference from the standard across the melting temperatures.

25 -26. (Cancelled)

27. (Currently Amended) The method of claim 2[5]4 further comprising the step of temperature shifting the melting curves by superimposing a portion of each curve.

28. (Previously Presented) The method of claim 27 further comprising the step of plotting the fluorescence difference between the temperature shifted curves, wherein the melting curve is selected as the standard and is plotted as standard across temperatures and the melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures.

29. (Cancelled)

30 (Previously Presented) The method of claim 25 wherein the dye is selected from the group consisting of PO-PRO™-1, JO-PRO™-1, BO-PRO™-1, SYTO® 45, POPO™-3, SYTO® 12, TOTO™-3, SYTOX® Blue, YOYO®-3, SYTO® 43, SYTO® 11, G5, H5, D6, E6, P6, R6, Y6, Z6, and D8.

31-48. (Cancelled)

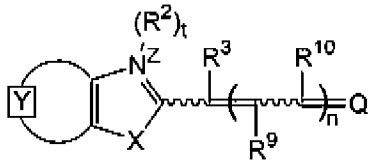
49. (Previously Presented) A method of PCR analysis comprising the steps of:

mixing a dsDNA binding dye having a percent saturation of at least 50% with a sample comprising a target nucleic acid and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye, and

monitoring fluorescence of the dsDNA binding dye, wherein the monitoring step comprises melting the amplified target nucleic acid to generate a melting curve, and

identifying the genotype using a shape of the melting curve, and wherein the dsDNA binding dye is a compound having the formula:



wherein

the moiety Y represents an optionally-substituted fused monocyclic or polycyclic aromatic ring or an optionally-substituted fused monocyclic or polycyclic nitrogen-containing heteroaromatic ring;

X is oxygen, sulfur, selenium, tellurium or a moiety selected from $C(CH_3)_2$ and NR^1, where R^1 is hydrogen or C_{1-6} alkyl;

R^2 is selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, aryl, aryl(C_{1-2} alkyl), hydroxyalkyl, alkoxyalkyl, aminoalkyl, mono and dialkylaminoalkyl, trialkylammoniumalkyl, alkylenecarboxylate, alkylenecarboxamide, alkylenesulfonate, alkylsulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties;

t = 0 or 1;

Z is a charge selected from 0 or 1;

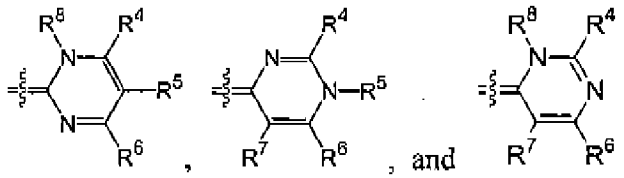
R^3 is selected from the group consisting of hydrogen, C_{1-6} alkyl, and -C(O)Ph;

R^9 and R^{10} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl;

n = 0, 1, or 2;

~~~ indicates a single bond that is in a tautomeric relationship with an adjacent double bond, and

Q is an heterocycle selected from the group of structures consisting of:



wherein R^4, R^5, R^6, R^7, and R^8 are independently selected from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, alkenyl, polyalkenyl,
~~~

alkynyl, polyalkynyl, alkenylalkynyl, aryl, heteroaryl, alkoxy, alkylthio, and dialkylamino, each of which may be optionally substituted; an acyclic heteroatom-containing moiety or a cyclic heteroatom-containing moiety; a BRIDGE-DYE; and a reactive group; each of which optionally includes a quaternary ammonium moiety.

50. (Previously Presented) The method of claim 49 wherein the moiety Y represents an optionally-substituted fused monocyclic or polycyclic aromatic ring selected from the group consisting of optionally substituted benzo, optionally substituted pyridino, and optionally substituted naphtho; and X is oxygen or sulfur.

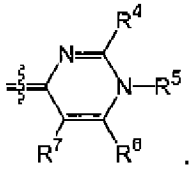
51. (Previously Presented) The method of claim 49 wherein the moiety Y represents a benzo or a naphtho having a substituent selected from the group consisting of halo, alkyl, amino, monoalkylamino, dialkylamino, alkylsulfonyl, haloalkylsulfonyl, and optionally substituted phenylsulfonyl.

52. (Cancelled)

53. (Previously Presented) The method of claim 49 wherein R^2 is selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, aryl, aryl(C_{1-2} alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, alkylsulfonate, alkylenesulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties.

54. (Cancelled)

55. (Previously Presented) The method of claim 49 wherein Q is the heterocycle:



56. (Previously Presented) The method of claim 49 wherein R^4, R^5, R^6, R^7, and R^8 are independently selected from the group consisting of hydrogen, halogen, thiol, alkylthio, alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

57. (Previously Presented) The method of claim 49 wherein t is 1, n = 0, and at least one of R^4, R^5, R^6, R^7, and R^8 is selected from the group consisting of halogen, thiol, alkylthio, C_{2-6} alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

58. (Previously Presented) The method d of claim 57 wherein R^5 is selected from the group consisting of halogen, thiol, C_{2-6} alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

59-60. (Cancelled)

61. (Previously Presented) The method of claim 57 wherein R^3, R^9, and R^{10} are each hydrogen; and R^2 is selected from the group consisting of C_{1-6} alkyl, aryl, aryl(C_{1-2} alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, alkylsulfonate, and alkylenesulfonate.

62-82. (Cancelled)

83. (Currently amended) A method of PCR analysis comprising the steps of:

providing a mixture of a dsDNA binding dye, a target nucleic acid, and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye having at least 90% saturation,

monitoring fluorescence of the dsDNA binding dye,

generating a melting curve for the target nucleic acid.

normalizing magnitude differences of the melting curve,

repeating the providing, amplifying, normalizing, and generating steps with at least one additional target nucleic acid,

comparing the magnitude-difference-normalized melting curves, and

~~The method of claim 45 further comprising the step of~~ plotting the fluorescence difference between the magnitude difference normalized curves, wherein the melting curve of one selected target nucleic acid is selected as the standard and is plotted as standard across temperatures and the melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures.

84. (Currently Amended) A method of PCR analysis comprising the steps of:

providing a mixture of a dsDNA binding dye, a target nucleic acid, and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye,

monitoring fluorescence of the dsDNA binding dye,

generating a melting curve for the target nucleic acid,

normalizing magnitude differences of the melting curve,

repeating the providing, amplifying, normalizing, and generating steps with at least one additional target nucleic acid,

comparing the magnitude-difference-normalized melting curves, and

plotting the fluorescence difference between the magnitude difference normalized curves, wherein the melting curve of one selected target nucleic acid is selected as the standard and is plotted as standard across temperatures and the melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures ~~The method of claim 83~~ wherein the standard is plotted as zero across all temperatures.

85. (Previously Presented) The method of claim 47 further comprising the step of plotting the fluorescence difference between the temperature shifted curves, wherein the melting curve is selected as the standard and is plotted as standard across temperatures and the shifted melting curve for each additional target nucleic acid is plotted as a difference from the standard across the temperatures.

86. (Cancelled)